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Pentahymena corticicola nov. gen., nov. spec., a New Colpodid Ciliate (Protozoa, Ciliophora) from Bark of *Acacia* Trees in Costa Rica

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Summary: *Pentahymena corticicola* nov. gen., nov. spec. was discovered in the bark of an *Acacia* tree from the Santa Rosa National Park in Costa Rica, Central America. Its morphology and infraciliature were studied in live cells and in specimens impregnated with silver nitrate and silver carbonate. The new genus, *Pentahymena*, belongs to the family Jaroschiidae and is unique in having five differently shaped oral structures and a distinct preoral suture containing many brick-shaped adoral organelles. The new species, *P. corticicola*, measures 130–160 x 70–100 µm and has an ellipsoid macronucleus, several micronuclei, and a contractile vacuole with collecting canals at the posterior end. About 55 somatic kineties commence around the oral apparatus and along the preoral suture and course spirally posteriad. The oral apparatus is in the anterior ventral third. The vestibular opening is slit-like and obliquely orientated to the longitudinal axis of the cell. The vestibulum is deep and narrow and contains the highly complicated oral infraciliature. On the right vestibular slope are about 8 densely ciliated vestibular kineties, on the left and in the preoral suture are approximately 25 small, brick-shaped adoral organelles. On the inner portion of the vestibular wall are two large ciliary fields composed of many short, tightly spaced kineties; the right field is hook-shaped and longer than the slightly crescentic left field. The anterior end of the postoral kineties is sharply bent back and densely ciliated, forming membranoid structures along the left oral ciliary field. 33 species of ciliates, which occurred together with *P. corticicola*, are new for the fauna of Costa Rica and listed in the ecology section.

Key Words: Bark fauna; Colpodea; Jaroschiidae; Central America; Infraciliature.

Introduction

The ciliate fauna of the bark of trees is poorly known. The first systematic studies date back to GELLÉRT (1942, 1950) who discovered several new species and even a new genus, *Cirrophrya*, in bark from *Picea excelsa*. *Cirrophrya* is a colpodid ciliate with unique adhesive organelles which secrete a glutinous substance used to adhere the ciliate to the substrate (FOISSNER 1993; GELLÉRT 1950). Recent electron microscopic investigations showed that these little feet contain microsporidian parasites (FOISSNER & FOISSNER 1993).

More recently, BLATTERER & FOISSNER (1988) and FOISSNER (1993, 1994) described six new genera and species from bark of trees in Australia and Hawaii. In this paper I report on another new corticolous species, *Pentahymena corticicola*, discovered in the bark of an *Acacia* tree in Costa Rica, Central America.

Material and Methods

Pentahymena corticicola was collected on 13. 2. 1991 from bark of an *Acacia* tree growing by the ranch house "La Casona" in the Santa Rosa National Park, Costa Rica (W 85°40′ N 10°50′). This area harbours a tropical dry forest (1600 mm rainfall, 6 months dry season) with an

incredible wealth and variety of plants and wild life, viz. 240 species of trees and shrubs, 115 species of mammals, 253 of birds, 100 of amphibians and reptiles, over 10,000 of insects, including 3410 species of moths and butterflies (BOZA 1988). The old, large *Acacia* tree had a thick, chapped bark, the outer and dry layer of which was collected.

In the laboratory, the dry bark was saturated with distilled water according to the non-flooded petri dish method (FOISSNER 1992a). The rewetted bark had pH 7.1. *Pentahymena corticicola* appeared 10 days after rewetting. Only few individuals were found. Attempts to establish pure cultures failed and all data thus refer to material collected from the raw culture.

Cells were studied in vivo using a high-power oil immersion objective and differential interference contrast optics (FOISSNER 1992b). Silver nitrate and silver carbonate were used to reveal the silverline system, the infraciliature, and other cytological details (FOISSNER 1991). Counts and measurements on silvered specimens were performed at a magnification of X 1000. In vivo measurements were conducted at a magnification of X 250–1000. Although these provide only rough estimates, it is convenient to give such data as specimens usually shrink in preparations or may even contract during fixation. Standard deviation and coefficient of variation were calculated according to statistics textbooks. Drawings of impregnated cells were made with a camera lucida.

Terminology is according to the monograph by FOISSNER (1993).

Pentahymena nov. gen.

Diagnosis: Medium sized Jaroschiidae with five differently shaped oral ciliary fields and distinct preoral suture containing many brick-shaped adoral organelles.

Type species: *Pentahymena corticicola* nov. spec.

Derivatio nominis: Composite of "penta" (five) and "hymen" (membrane); both Greek. Feminine. The name refers to the five membranous structures forming the oral infraciliature.

Comparison with related genera: The new genus apparently belongs to the Jaroschiidae, an unusual group of bryometopid colpodids, which diagnosed FOISSNER (1993) as follows: "Moderately small to medium sized, completely ciliated Bryometopida. No postoral suture. Oral apparatus very complicated, consists of more than 3 differently structured elements. At right and proximal slope of vestibulum several vestibular kineties and paroral membrane-like structures comprising many short ciliary rows. At left vestibular slope several brick-shaped adoral organelles. Silverline system presumably very tightly meshed". Unlike *Jaroschia sumptuosa* FOISSNER, 1993, *P. corticicola* has a distinct preoral suture containing brick-shaped adoral organelles. This resembles the colpodid order Bryophryida. Thus, it can not be excluded that *Pentahymena* and *Jaroschia* belong to this order. Unfortunately, the type species of the genus *Bryophrya* is still insufficiently known. Thus, a proper classification of the Bryophryidae and Jaroschiidae is not yet possible. Both, *Pentahymena* and *Jaroschia* have been found in bark and are probably restricted to this biotope.

Description of *Pentahymena corticicola* nov. spec.

Diagnosis: In vivo 130–160 x 70–100 µm, reniform. Single ellipsoid macronucleus, several micronuclei. Contractile vacuole with collecting canals at posterior end. About 55 distinctly spiralling somatic kineties. Vestibulum deep and narrow, on right slope about 8 vestibular kineties, on left slope and in preoral suture approximately 25 brick-shaped adoral organelles. Right oral ciliary field hook-shaped and longer than slightly crescentic left field, both composed of many tightly spaced, short kineties. Anterior end of postoral kineties sharply bent back and densely ciliated, forming membranoid structures along left oral ciliary field.

Type location: Bark of an *Acacia* tree by the ranch house "La Casona" in the Santa Rosa National Park, Costa Rica, W 85°40′ N 10°50′.

Type specimens: Two holotypes and two paratypes of *P. corticicola* as four slides of silver nitrate (CHATTON-LWOFF and KLEIN technique) impregnated cells have been deposited in the collection of microscope slides of the Oberösterreichische Landesmuseum in Linz, Austria.

Derivatio nominis: "corticicola" (Latin, living in bark) refers to the biotope.

Description: Only few, mediocrely impregnated specimens were found in the Chatton-Lwoff slides. Material was to scanty for protargol impregnation. Morphometry is thus incomplete and the description is based mainly on cells studied in vivo and impregnated with silver carbonate.

Shape resembling *Colpoda maupasi* and/or *Bryophrya* spp., viz. right and dorsal side convex, left and ventral side slightly indented at oral apparatus, both ends broadly rounded (Fig. 1). Inconspicuously flattened dorso-ventrally. Prepared cells often broadly spindle-shaped (Fig. 4). Macronucleus distinctly ellipsoid to

Table 1. Morphometric data from *Pentahymena corticicola*[1]).

Character	$\bar{x}$	M	SD	$SD_{\bar{x}}$	CV	Min	Max	n
Body, length	137.8	135.0	8.5	2.7	6.1	125	153	10
Body, width	77.5	76.0	7.9	2.5	10.2	70	95	10
Distance anterior end to vestibular opening	13.4	15.0	2.1	0.8	15.7	10	15	7
Distance anterior end to proximal edge of vestibulum	55.7	55.0	3.1	1.2	5.7	50	60	7
Distance anterior end to macronucleus	65.4	65.0	9.9	4.4	15.2	52	75	5
Macronucleus, length	41.0	45.0	8.0	3.6	19.6	32	50	5
Macronucleus, width	14.2	15.0	1.1	0.5	7.7	13	15	5
Excretory pore, diameter	3.5	3.5	0.5	0.2	15.6	3	4	6
Somatic kineties, number	54.0	50.0	6.5	2.9	12.1	50	65	5

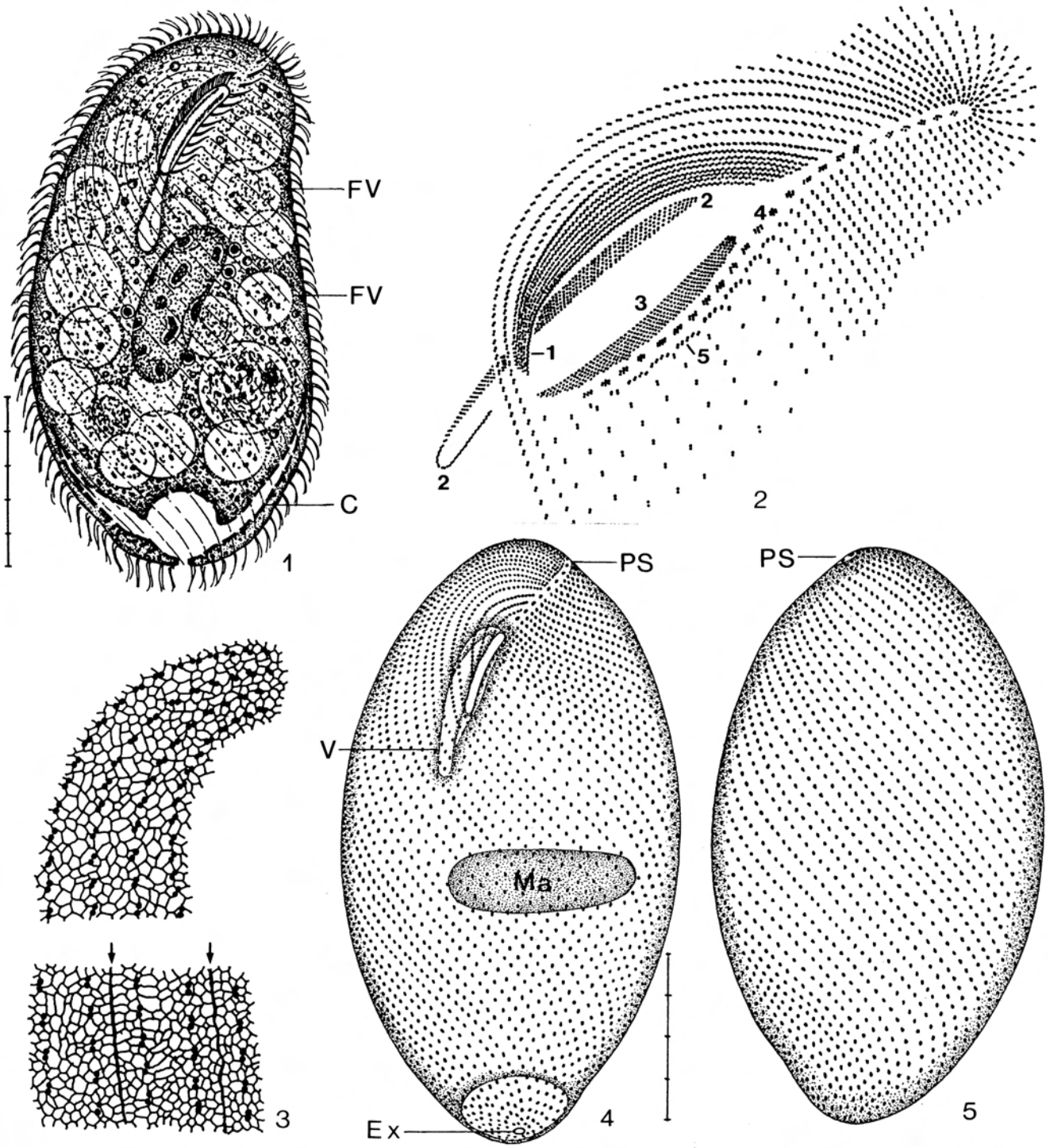
[1]) Data based on 10 silver nitrate (CHATTON-LWOFF technique) impregnated cells found in the slides. Measurements in µm. CV = coefficient of variation in %, M = median, Max = maximum, Min = minimum, n = number of specimens investigated, SD = standard deviation, $SD_{\bar{x}}$ = standard deviation of the mean, $\bar{x}$ = arithmetic mean.

slightly reniform, in middle third of cell. About 4 micronuclei near macronucleus, in vivo 4 µm in diameter and surrounded by distinct membrane (Fig. 1). Contractile vacuole with 2 collecting canals at posterior end, discharges via tubular excretory pore in centre of posterior pole (Figs. 1, 4). Cortex slightly furrowed by somatic kineties, flexible, contains plate-like layer of disc-shaped mitochondria about 1.2 µm in size (Fig. 6). No extrusomes recognizable in vivo and in silver carbonate stains. Cytoplasm colourless, postorally usually crammed with (i) up to 50 µm sized food vacuoles having loose content, some even appear empty making cytoplasm looking strongly vacuolated (Figs. 1, 7); (ii) many greasily shining granules 1–3 µm in diameter, and (iii) some yellowish, about 2 x 1 µm sized crystals concentrated in posterior end around contractile vacuole. Feeds on ciliates (e. g. *Colpoda*), which are quickly digested. Moves slowly, glides and/or rotates about longitudinal axis.

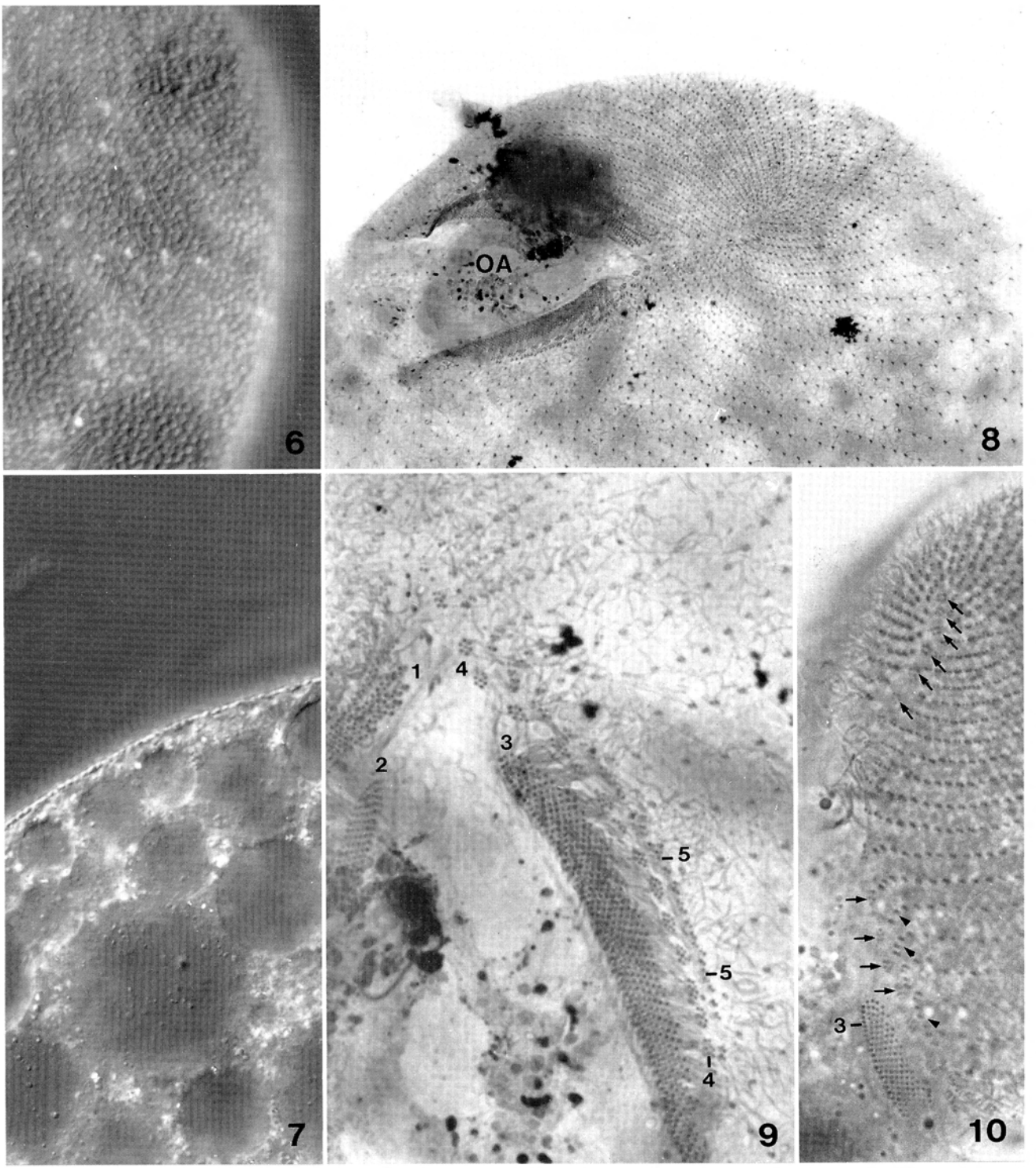
Somatic kineties distinctly spiralling, composed of slightly inclined, ciliated dikinetids. All commence around oral apparatus and along postoral suture, and most extend to posterior end of cell. Kineties originating at left oral ciliary fields slightly wider spaced than preoral ciliary rows (Figs. 2, 4, 5, 8, 9).

Oral apparatus in anterior ventral third. Vestibular opening slit-like, vestibulum deep and narrow, obliquely orientated to longitudinal axis of cell, contains oral ciliary fields except of those found in preoral suture (Fig. 1). Oral infraciliature very complicated, comprises 5 clearly distinguishable ciliary fields on vestibular walls and in preoral suture (Figs. 2, 4, 8–12, 14, 15). First (rightmost) oral ciliary field composed of about 8 vestibular kineties on right slope of vestibulum. Vestibular kineties consist of closely spaced dikinetids, producing heavily beating ciliary plate; dikinetids more loosely and zigzag-like arranged in anterior portion of kineties than in posterior one where they form very tightly spaced, slightly irregular rows. Some vestibular kineties may be shortened, but most end at proximal vestibular vertex and do not extend posteriad as normal somatic kineties. Second oral ciliary field on inner portion of right vestibular wall, consists of many short, oblique kineties forming long, hook-shaped structure extending from anterior edge of vestibular opening beyond its posterior vertex, i.e. into tubular portion of vestibulum; proximal portion curved back to vestibular opening and distinctly narrowed, i.e. composed of single row of dikinetids. Third oral ciliary field on inner portion of left vestibular wall, about as long as vestibular opening, i. e. shorter but broader than second ciliary field, slightly crescentic, left edge uneven because of different length of kineties. Fourth oral ciliary field on exterior portion of left vestibular wall and in preoral suture, consists of about 25 brick-shaped adoral organelles extending from proximal vertex of vestibular opening to anterior pole of cell; individual organelles composed of 2–5 kineties with 2–3 basal bodies each. Fifth oral ciliary field formed by anterior ends of postoral kineties which bend back in acute angle and polymerize dikinetids to membranoid structures.

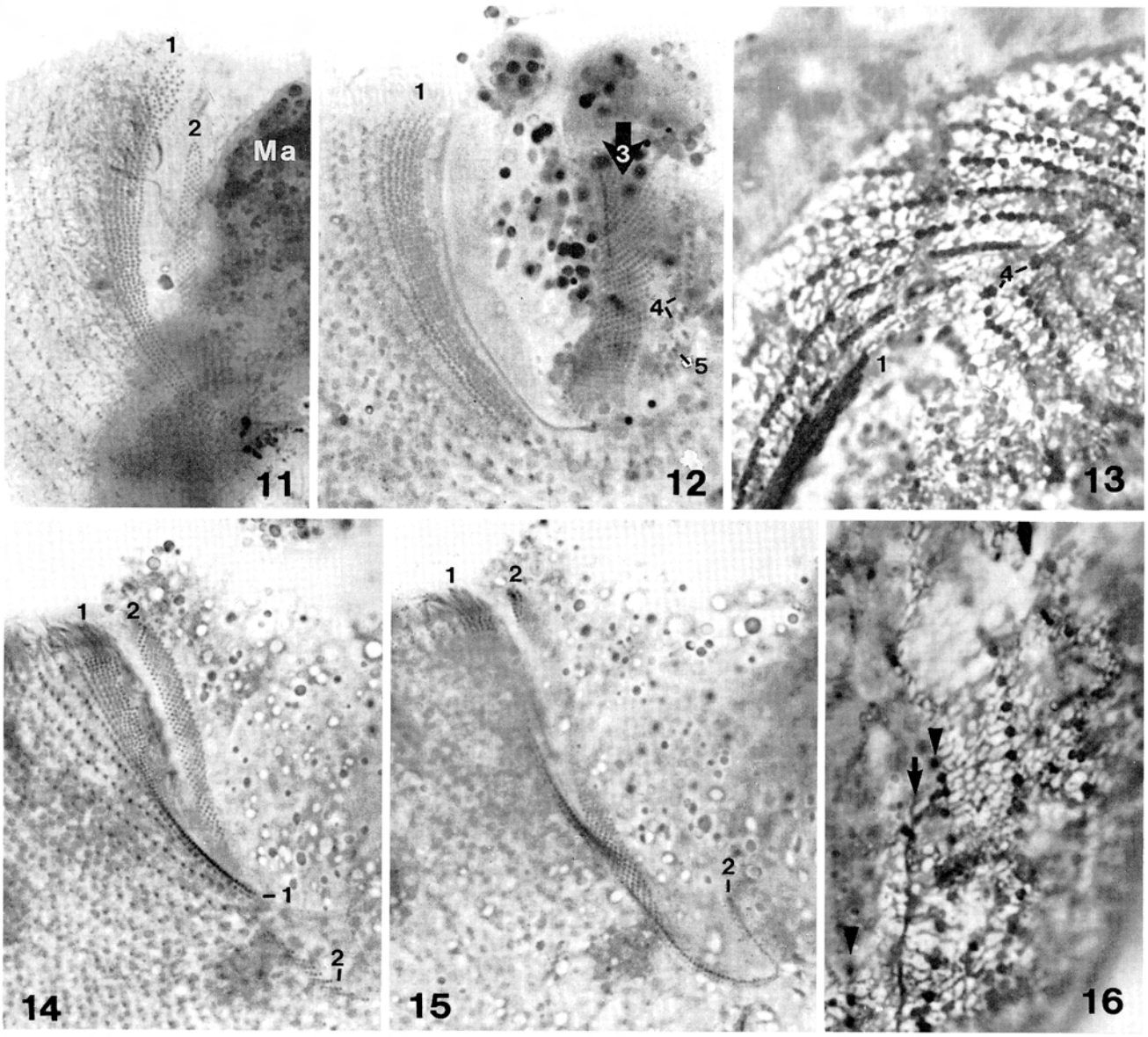
Silverline system tightly and irregularly meshed, in posterior body portion with distinct argyrophilic line between some somatic kineties (Figs. 3, 13, 16).



Figs.1–5. *Pentahymena corticicola* from life (Fig. 1), after silver carbonate (Fig. 2), dry (KLEIN) silver nitrate (Fig. 3), and wet (CHATTON-LWOFF) silver nitrate impregnation (Figs. 4, 5). – 1. Ventral view of well-nourished specimen. C = canal of contractile vacuole, FV = food vacuoles. Scale bar division = 10 µm. – 2. Diagram of oral infraciliature; numbers designate individual components: 1 = vestibular kineties, 2 = right oral ciliary field, 3 = left oral ciliary field, 4 = adoral organelles along left oral ciliary field and in preoral suture, 5 = membranoid structures formed by polymerized dikinetids at anterior end of postoral kineties. – 3. Silverline system right of oral apparatus and near posterior end of cell. Arrows mark straight silverlines between somatic kineties. – 4, 5. Infraciliature of ventral and dorsal side. Ex = excretory pore of contractile vacuole, Ma = macronucleus, PS = preoral suture, V = vestibulum. Scale bar division = 10 µm.



Figs. 6–10. *Pentahymena corticicola* from life (Figs. 6, 7) and after silver carbonate impregnation (Figs. 8–10). Figures purposely without scale bars since the applied techniques (squashed, unmounted specimens) lead to inavoidable distortions of cells. – 6. Surface view showing tightly spaced mitochondria forming plate-like layer beneath cortex. – 7. The cytoplasm contains many food vacuoles with loose content. – 8. Infraciliature of anterior pole area. 50 somatic kineties, which commence around the oral apparatus (OA) and preoral suture, are recognizable (for details see Figs. 9–12, 14, 15). – 9. Oral infraciliature of specimen shown in Fig. 8. Numbers designate individual components (cp. Fig. 2): 1 = anterior end of vestibular kineties, 2 = anterior end of right oral ciliary field, 3 = left oral ciliary field, 4 = adoral organelles along left oral ciliary field and in preoral suture, 5 = membranoid structures formed by polymerized dikinetids at anterior end of postoral kineties. – 10. Anterior pole region showing adoral organelles (arrows) in preoral suture and membranoid structures (arrowheads) formed by polymerized dikinetids at anterior end of postoral kineties.



Figs. 11–16. *Pentahymena corticicola* after silver carbonate impregnation (Figs. 11, 12, 14, 15) and dry (KLEIN) silver nitrate impregnation (Figs. 13, 16). Figures purposely without scale bars since the applied techniques lead to inavoidable distortions of cells. – 11, 12. Details of oral infraciliature; numbers designate individual components. – 13. Silverline system in anterior pole area. Numbers designate components of oral infraciliature. – 14, 15. Same specimen photographed at two focus levels to show hook-shaped right oral ciliary field (number 2). – 16. Silverline system in posterior body region. Arrow marks straight silverline between somatic kineties (arrowheads). Ma = macronucleus, 1 = vestibular kineties, 2 = right oral ciliary field, 3 = left oral ciliary field, 4 = adoral organelles along left oral ciliary field and in preoral suture, 5 = membranoid structures formed by polymerized dikinetids at anterior end of postoral kineties.

Comparison with related species: No other species have been found in the literature which could be identical with *P. corticicola*. Superficially, *Jaroschia sumptuosa* and *Bryophrya* spp. resemble *P. corticicola*, but their oral structures are clearly different, which is easily recognizable even in living cells (FOISSNER 1993).

Occurrence and ecology: As yet found only at type location, together with the following species which are also new for the fauna of Costa Rica: *Avestina* sp. (few specimens; a large, very likely new species), *Blepharisma* sp. (a bluish, possibly new species), *Colpoda ecaudata* (LIEBMANN, 1936), *C. inflata* (STOKES, 1885),

C. lucida GREEFF, 1888, *C. maupasi* ENRIQUES, 1908, *C. praestans* PENARD, 1922, *C. tripartita* KAHL, 1931, *Cyclidium muscicola* KAHL, 1931, *Cyrtohymena candens* (KAHL, 1932), *Drepanomonas revoluta* PENARD, 1922, *D. sphagni* KAHL, 1931, *Enchelyodon* sp. (single specimen), *Euplotes muscicola* KAHL, 1932, *Epispathidium papilliferum* (KAHL, 1930), *Epispathidium* sp. (very likely a new species), *Frontonia depressa* (STOKES, 1886), *Gonostomum affine* (STEIN, 1859), *Halteria grandinella* (MÜLLER, 1773), *Hemisincirra inquieta* HEMBERGER, 1985, *Holosticha muscorum* (KAHL, 1932), *Kahlilembus fusiformis* (KAHL, 1926), *Leptopharynx costatus* MERMOD, 1914, *Lepidotrachelophyllum* sp. (very likely a new species), *Oxytricha granulifera* FOISSNER & ADAM, 1983, *Phacodinium metchnicoffi* (CERTES, 1891), *Platyophrya vorax* KAHL, 1926, *Protospathidium bonneti* (BUITKAMP, 1977), *Pseudouroleptus* sp. (very likely a new species), *Sathrophilus muscorum* (KAHL, 1931), *Tachysoma humicola* GELLÉRT, 1957, *Tetrahymena rostrata* (KAHL, 1926), *Vorticella astyliformis* FOISSNER, 1981.

This list contains 33 species 5 of which are probably new, demonstrating our ignorance about the bark fauna.

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